Supercritical impregnation of polymer matrices spatially confined in microcontainers for oral drug delivery: effect of temperature, pressure and time

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Abstract

The present study is aimed to enhance the oral bioavailability of ketoprofen by inserting it into the matrix of poly(vinylpyrrolidone) (PVP) K10, a water soluble polymer, spatially confined into microcontainers, by means of supercritical CO₂-aided impregnation. Microcontainers are cylindrical reservoirs, with typical sizes in the micrometer range, with a cavity open on one side, where the drug formulation is loaded. Differently to traditional tablets, microcontainers have a higher surface area per unit volume, and release the drug only in one direction. This design is meant to enhance the absorption of problematic drugs, like those with poor solubility in water. In a previous study we introduced a novel technique for drug loading of microcontainers, based on inkjet printing and supercritical impregnation (SCI). We showed that SCI produces accurate and reproducible drug loading for large arrays of microcontainers. In the attempt of enhancing the throughput of the loading methods, we propose the replacement of polymer inkjet printing with an easier manual compression of the PVP powder into the microcontainers. As the second step, the polymer powder filled-microcontainers were submitted to SCI. The separate role of different impregnation parameters (temperature, pressure, time, drug concentration in the supercritical phase) was elucidated with respect to the loading capacity. The microcontainer filling was observed by means of optical macroimaging, X-ray microtomography and scanning electron microscopy. The physical state of the drug was investigated by means of Raman spectroscopy and compared with selected representative PVP-ketoprofen physical mixtures. Finally, the drug loading was estimated by means of in vitro dissolution tests.

The characterization study shows that the present loading method is a valuable alternative to the one previously described. The drug loading can be controlled with high accuracy and reproducibility and the impregnated drug is in amorphous state. These results demonstrate that SCI can be used as a high throughput loading technique for microfabricated devices for oral drug delivery.
1. Introduction

Among the different drug delivery routes oral administration is still the most preferred one for its simplicity, minimal invasiveness, and high patient compliance. Nevertheless, the human digestive system presents a sequence of physiological barriers which drastically reduce the bioavailability of many active pharmaceutical ingredients (API): enzymatic degradation, hydrolysis in the gastric acidic environment, thick mucus layer covering the intestinal mucosa, selective transport action of peptide receptors in the epithelial cells [1]. Such unfavorable conditions are affecting APIs that exhibit low solubility in water. The solid state properties of drugs have a strong influence on their solubility. Whilst amorphous drug candidates exhibit an enhanced solubility and dissolution rate compared to their crystalline counterparts, amorphous forms often suffer from rather short thermodynamic stability and they spontaneously tend to crystallize [2]. As a result, stabilization of amorphous forms is necessary in order to preserve the abovementioned advantages [3]. Physical stabilization of the amorphous form can be achieved by the addition of a polymeric carrier wherein the drug is confined in supramolecular domains or even molecularly dispersed [4]. Together with the solid state properties, the solubility and dissolution rate can be improved by reducing the particle size of the formulation [5]. Among the several formulation approaches [6] supercritical fluid based technology is a promising technique to produce micro- and nanoparticulated systems with high drug dispersion and enhanced stability and dissolution properties [7].

Beside the properties of the drug formulation, an important role in the therapeutic performance is often played by the design of the administration form. Conventional oral dosage forms like tablets provide a omni-directional drug release through their limited interfacial area when exposed to the physiological fluids. Furthermore, drug release from tablets is often slow compared to the peristaltic flow in the GI tract, which is the most permeable tissue for drug absorption. As a result, a large amount of API is not delivered, and patients need to ingest multiple drug doses to receive the desired therapeutic benefit. This increases the occurrence of potentially harmful side effects in patients [8].

In the last 10 years advances in field of micro- and nanofabrication have allowed the development of alternative drug delivery systems [9]. In particular there has been an increasing interest in microfabricated devices based on the concept of microcontainers [10]. A microcontainer is a reservoir with the typical dimensions falling in the micrometer range, composed by a non-permeable and inert shell and a cavity for the drug formulation open on one side from which the drug is released undirectionally. Several groups developed microcontainers in different shapes, materials and designs, where fabrication is typically performed on flat silicon chips. By virtue of its asymmetric shape and by applying appropriate bioadhesive coatings on microcontainers Ainslie et al. [11] showed an increased intestinal retention time for these microdevices and an enhanced bioavailability for a model poorly water soluble drug. One of the big challenges in the fabrication of microcontainers concerns the drug loading step. In a previous work [12], we showed the fabrication of cylindrical microcontainers, fabricated with the epoxy resin SU-8. The microwells were filled with poly(vinylpyrrolidone) (PVP) by inkjet printing. This method showed high accuracy and a minimal waste of materials. In a more recent work [13] we proposed the combination of inkjet printing and supercritical technology to impregnate the polymer-filled microcontainers with ketoprofen, a poorly water soluble drug. We demonstrated that the supercritical impregnation technique allows a highly accurate and reproducible drug loading of large arrays of microcontainers. In an attempt to enhance the throughput of this loading method, we propose a simplified variant of our previous work. Here the polymer printing was replaced by an easier filling method, consisting of a manual compression of the polymer powder onto the microcontainer. Later, the powder filled-containers were submitted to supercritical impregnation (SCI) as previously described [13]. This process modification enabled a significant reduction in the sample
preparation time and made it possible to investigate the effect of temperature, pressure and
impregnation times on different aspects concerning the drug loading in more detail.
The loading procedure was characterized with different techniques. X-ray microtomography was
used to measure the level of filling and the polymer morphology of the polymer powder in the
microreservoir cavities before and after the SCI. In addition, microcontainers were visualized by
scanning electron microscopy (SEM) to observe the effect of the impregnation parameters on the
polymer morphology. Raman spectroscopy was used to investigate the drug solid state in the
impregnated matrices and drug-polymer interactions. Finally, in vitro dissolution tests were
carried out and the total drug loading was estimated. The results showed that the replacement of
inkjet printing with the powder filling method enhances the throughput of the microcontainer
loading technique without compromising the accuracy or the reproducibility of the whole loading
process.

2. Materials and methods

2.1 Fabrication of SU-8 microcontainers

2.1.1 Materials and fabrication of microcontainers

Silicon wafers (4-inch b100N n-type) were supplied by Okmetic (Vantaa, Finland). SU-8 2075 and
SU-8 developer were purchased from Microresist Technology GmbH (Berlin, Germany).
Cylindrical microcontainers were fabricated with a similar procedure as previously described [12].
The microwells were fabricated with the epoxy-based photoresist SU-8 on silicon wafers arranged
in a squared chip containing an array of 25x25 microcontainers. A microcontainer has a cavity of
approximately 300 µm in diameter and 270 µm in depth and an approximated volume of 18 nL.
After the fabrication the wafer was cut into square chips containing 625 microcontainers (DISCO
DAD 321, Automatic Dicing Saw).

2.2 Drug loading of microcontainers

2.2.1 Materials

Ketoprofen (98%, racemate) and polyvinylpyrrolidone (PVP K10, Mw 10,000) were supplied by
Sigma Aldrich. Carbon dioxide was supplied by SIAD (99% purity). Ketoprofen is a nonsteroidal
anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. In several
pharmacopeias ketoprofen is considered as practically insoluble in water [14]. Its solubility in
pure water at room temperature (22-24°C) was reported to be 0.010 mg/mL [15]. Thus,
ketoprofen is classified as a class II active principle in the biopharmaceutical classification system
(BCS): It exhibits low aqueous solubility and a high intestinal permeability.

Figure 1: Chemical structure of (a) ketoprofen, (b) monomer of poly (vinyl pyrrolidone) (PVP).
2.2.2 Filling with poly(vinylpyrrolidone) PVP powder

Microcontainers were filled with PVP powder with the following procedure: the powder was deposited and compacted with a spatula onto the microwells and the residual amount, placed in between containers, was blown away by means of a pressurized air. The chip was weighed before and after filling and the average PVP weight per chip was estimated. The level of microcontainer filling was measured by X-ray microtomography in different positions of the chip.

2.2.3 Supercritical impregnation of polymer filled-microcontainers

After the powder deposition the drug was loaded into the polymer-filled microdevices by means of supercritical carbon dioxide impregnation. Figure 1.2 depicts a schematic of the high pressure setup used in the impregnation experiments. The operation was performed in a 100 mL reactor (KM 20-05 autoclave NWA, Germany) equipped with a magnetic stirrer drive (Mrk MINI 100) assembled in a sealed housing directly threading into the reactor head. During the impregnation experiments the reactor was fed with liquid CO\(_2\) through an on/off valve (V1) by a high pressure pump (PM-101NWA, NWA, Germany). At the end of the experiments the reactor was slowly emptied through an on/off purge valve followed by a lamination valve (V2) and the outlet stream was connected to a deflux flask where the gaseous CO\(_2\) was bubbled through an ethanol solution (95%) and the ketoprofen concentration was measured. The resulting drug solution was made up to defined volumes and the drug content was estimated by UV spectroscopy measurements (Thermo Scientific Evolution 60 - Thermo Fisher Scientific Inc.). Finally, the drug free-CO\(_2\) stream was connected to a flowmeter (SIM Brunt, Italy).

![Figure 2: Schematic of the high pressure plant. F\(_1\) and V\(_1\) indicate 0.5 µm filters and on/off valves.](image)

The impregnation experiments were carried out in batch conditions with defined steady-state values of CO\(_2\) pressure, temperature and stirring velocity. In each batch, weighted amounts of crystalline ketoprofen powder and one chip were placed in separate compartments of a sample carrier placed at the bottom of the reactor. In order to avoid damage to the microcontainers during the chamber filling with the supercritical fluid, the back of the chips were glued onto the sample carrier with a carbon pad tape. The reactor was then sealed and heated to the defined temperature. When the set point temperature was achieved, the inlet valve was gradually opened and the chamber was slowly filled with CO\(_2\) in order to maintain isothermal conditions and avoid turbulence within the reactor, which could result in detachment of the chip and damaging of the microcontainers. When the desired pressure was achieved, the inlet valve was closed and the stirrer was switched on. During the experiment the supercritical CO\(_2\) phase dissolved the solid drug powder and swelled the polymer within the microcontainers. The drug was therefore physically
conveyed and loaded in the polymer matrices. The impregnations were carried out at a fluid pressure of 100 and 200 bar, temperatures of 40, 50 and 60°C and durations of 1 and 4 hours with a stirring velocity of 20 rpm, respectively.

In the experiments run at 100 bar the drug was dissolved under saturation conditions according to the solubility values of ketoprofen in supercritical CO$_2$ reported by Macnaughton et al. [16] at the chosen conditions of temperature. In this group of experiments the temperature and time were modified from batch to batch. Because of the batch conditions, the drug concentration could not be kept at solubility value over time. In contrast, in the tests performed at 200 bar a fixed amount of ketoprofen was dissolved, corresponding to the saturation concentration of the compound at 200 bar and 40°C. At 200 bar and 50°C and 60°C the amount of drug was dissolved under saturation conditions, as the solubility values are higher at these temperatures. In the experiment performed at 200 bar, the exclusive effect of temperature and time on the microcontainer impregnation was studied. At the end of the experiment the reactor was depressurized at a controlled rate for approximately 2 and 3 hours when the operating pressures were 100 and 200 bar respectively. After the experiments the chips were stored in a desiccator until further analysis were performed. In Table 1 the operating conditions of the experiments are shown.

Table 1: Mass of ketoprofen [mg] dissolved in the different experimental conditions

<table>
<thead>
<tr>
<th>Pressure [bar]</th>
<th>Temperature [°C]</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td>4.83</td>
<td>2.15</td>
<td>1.31</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>14.1</td>
<td>14.1</td>
<td>14.1</td>
</tr>
</tbody>
</table>

2.3 Characterization methods

2.3.1 X-ray microtomography (XμCT)

For the XμCT measurements a Skyscan 1172/F instrument (Skyscan, Kontich, Belgium, control software v1.5.13) was used. A source voltage of 60 kV and current of 165 μA were used together with a 0.5 mm thick Al filter to attenuate the high energy X-rays. For each sample 780 shadow images were acquired over 180° of rotation. The resulting data acquisition time was 4 h for each sample. Reconstruction of the cross-section images was performed using the program NRecon+GPUReconServer (Skyscan, beta v1.6.5) on a single PC using GPU accelerated reconstruction (Windows 7 64-bit workstation, 2 Intel Xeon X5647 with 4 cores each, 48 GB RAM, NVIDIA quadro 4000 with 256 cores). Image reconstruction using the Feldkamp algorithm [17] for cone beam geometry took about 30 min per sample and resulted in, depending on sample size, about 1450 slices of 2864 x 2876 pixels each (5.2 μm isotropic voxel size). Using the DataViewer (Skycan, v1.4.4) the tablet was rotated to align the microwells parallel to the x-axis.

2.3.2 Scanning electron microscopy (SEM)

The morphology of the impregnated microcontainers was examined using SEM. The investigations were carried out using a Nova600 NanoSEM from FEI (Eindhoven, the Netherlands). Imaging was performed in low-vacuum-mode at a pressure of 0.6 mbar and an operation voltage of 5 kV. Prior to examination, the samples were mounted onto metal stubs and were tilted by approx. 30 degrees.
2.3.3 Raman spectroscopy

As described in a previous study [13], Raman spectroscopy can be used to elucidate the presence of chemical interactions amongst drug molecules and between drug and polymer. The spectra from the impregnated microcontainers were collected using a Thermo Scientific Raman DXR microscope equipped with a frequency-stabilized single mode diode laser. The Raman signal collection time was 5 sec and the signal was averaged three times, using a 25 µm slit and a 1.0 µm diameter laser post. The Raman DXR microscope was coupled to a single grating spectrometer with a 5 cm−1 FWHM spectral resolution and a ±2 cm−1 wavenumber accuracy. All Raman spectra were recorded at a laser power of 10 mW, using a 10X objective lens and a laser excitation wavelength of 780 nm. In each sample 5 containers, located at different positions on the chip, were selected and analyzed. The intrinsic fluorescence background was fitted to a polynomial function and subtracted from the collected signal.

2.3.4 In vitro drug dissolution studies

Dissolution of PVP and ketoprofen from loaded microcontainers was determined in 10 ml DI water at 37 °C using a UV spectrophotometer μDISS profiler (Pion, USA). Individual chips were glued with carbon pads on teflon-coated magnets which stirred at 100 rpm. For the detection of ketoprofen, the wavelength was set to 259 nm. After dissolution, the microcontainers were observed with an optical microscope (Zeiss, Germany, 10x magnitude) to confirm complete emptying. The amount of drug loaded per chip was estimated by the final concentration measured in the dissolution test at 16 hours. Solubility of ketoprofen at 37 °C in aqueous solutions changes significantly with pH between 1 and 7 [14]. Nevertheless, even in the test with the highest drug loading, the pH of the dissolution medium drop below 6.5 as ketoprofen is a weak acid (pKₐ 4.5). Therefore, according to solubility data reported in the literature [14], the dissolution tests were performed in conditions below saturation that is without drug precipitation, which was also confirmed by the non-decreasing monotonic trend of the drug dissolution profiles (data not shown).

3. Results and discussion

3.1 Polymer filling of microcontainers and X-ray microtomography

A picture of arrays of microcontainers filled with PVP powder is shown in Figure 3. The powder filling required few seconds to be performed and resulted in an accurate deposition inside the cylindrical cavities of the microwells, with minimal residues in between, as representatively illustrated by the high color contrast between the dark silicon surface and the brilliant white of PVP powder. In Figure 4 a scanning electron microscopy image of microcontainers filled with PVP powder is reported. The polymer granules are compacted inside the microwells and this confinement prevents them from being removed by the air flow which is used to remove the loose powder after the filling step. Figure 5 shows the cross-sections that were reconstructed from the microtomography data of the XμCT measurements. Using this technique it is possible to measure the internal microstructure of the PVP powder within the containers and to visualise the effect of the impregnation on the particle morphology. The images clearly show that the microcontainers are filled with discrete PVP particles before impregnation and subsequently coalesce following treatment with supercritical CO₂.
Figure 3: Optical macro images of microcontainers filled with PVP powder by manual compaction: (a) detail of the chip corner, (b) arrays in the middle of the chip.

Figure 4: SEM images of microcontainers filled with polyvinylpyrrolidone before treatment with supercritical CO₂. The scalebar indicates 300 µm.

Figure 5: Non-destructive cross-sections acquired by XμCT through the microcontainers filled with polyvinylpyrrolidone before treatment with supercritical CO₂ (a) and after impregnation (b). The original grey scale images are shown together with a binary threshold image to highlight the change in polymer morphology.
3.2 Scanning Electron Microscopy of impregnated microcontainers

After the supercritical treatment, the microcontainers were analyzed by scanning electron microscopy to observe the effects of the impregnation on the polymer morphology. As shown in a previously [13], the SEM micrographs (see Figure 6) confirm that scCO$_2$ does not visible effects on the epoxy resin used to fabricate the microcontainers (SU-8) which maintain their size, the cylindrical shape and the adhesion to the silicon substrate. The supercritical fluid has instead a much more pronounced effect on PVP. At all the tested conditions, the morphology of PVP radically changes as the polymer particles undergo swelling and subsequent coalescence as confirmed by the XµCT images (Figure 5b). By comparing the SEM micrographs of microcontainers treated at different pressures it is clear that the volume of the polymer visibly increases with increasing CO$_2$ pressure. This can be explained by an increase of CO$_2$ uptake in PVP with pressure, in accordance with what was previously reported by Kikic and co-workers [18]. At high pressures, CO$_2$ has a well-known plasticizing effect on certain polymers [19,20]. The plasticization leads to a drop in the $T_g$, which for PVP K10 is around 87 °C, a temperature close to the operating temperature during impregnation. As a result, the polymer gets close to the glass-rubber transition. The increase of impregnation time in general leads to a higher extent of swelling of the matrix and to the appearance of holes. At 40 °C and 50 °C these holes got larger when the pressure was set at 200 bar, and this fact can be attributed to the release of CO$_2$ from the highly swollen matrices. At 200 bar and 50 °C small spots appear on the polymer surface. At 200 bar and 60 °C these particles clustered in bigger aggregates (see Figures 6(k) and 6(l)) on small number of containers in the chip. As scCO$_2$ acts as a plasticizer for polymers the viscosity of the CO$_2$ saturated matrix decreases during impregnation [34] causing spillages, in some cases, from the containers. This aspect is more pronounced during longer experiments.
Figure 6: Scanning electron microscopy images of polymer filled microcontainers impregnated with ketoprofen by supercritical CO₂ at different pressure, temperature and time. The scalebar indicates 300 µm.

3.3 Loading of ketoprofen into polymer filled-microcontainers

The solubility of ketoprofen in scCO₂ monotonically increases with the fluid density, and therefore with the fluid pressure at isothermal conditions [16]. Furthermore, for many active compounds under isobaric conditions the temperature influences the solubility according to the operating pressure and with respect to the crossover pressures. At the crossover points solubility isotherms overturn their trend [16]. The upper crossover pressure of ketoprofen in carbon dioxide was measured between 160 and 180 bar [18,21]. Below this pressure, such as at 100 bar, the drug solubility decreases with temperature. At higher pressures, such as at 200 bar, the effect of temperature is the opposite. The impregnation pressures were chosen on different positions towards the upper crossover point in order to explore the behavior of the supercritical phase on the drug loading. In figure 7 the resulting weight fraction of ketoprofen \( \omega_{\text{keto}} \) loaded in a chip containing 625 PVP filled-microcontainers is compared for the different impregnation conditions. Despite the simplicity of the method the overall accuracy of the loading is similar to the one previously reported where the polymer was deposited by inkjet printing [12].

In the tests carried out at 100 bar (Figure 7a) the drug was dissolved in saturation conditions. Here the loading is strongly dependent on the drug concentration in the supercritical phase, which was higher at 40°C and lower at 60°C. There is an increase in drug loading over time for any tested temperature meaning that after 1 hour the equilibrium is not yet attained. After 4 hours the drug weight fraction is approximately doubled at all temperatures. To investigate the partition equilibrium of the drug between the CO₂ and the polymer phases an impregnation experiment was carried out at 40°C for 24 hours. The attained drug fraction was 0.27, which is very close to the loading achieved after 4 hours. In the experiments at 200 bar, the impregnation is enhanced compared to experiments at 100 bar as a result of the combined effect of the higher amount of drug dissolved (see table 1) and the density of the fluid at this pressure. However, at 200 bar the loading increased with the temperature which was unexpected, since at this pressure a rise of temperature leads to an increase of drug solubility and therefore a less favorable drug partition in the polymer matrix. Moreover, from 40 °C to 60 °C the fluid density decreases as shown in the profiles in Figure 8, where the loaded API weights are plotted as a function of the CO₂ density. These latter results can be explained by considering that the effect of temperature on drug-scCO₂ diffusivity prevails over the reduced fluid density. A temperature increase also results in enhanced mobility of the polymer chains, which is suggested by the visible swelling observed in Figures 6(d), 6(h) and 6(l). In contrast to the results at 100 bar, at 200 bar more limited loading gains for impregnation time were achieved. This might be due to the proximity of the drug concentration in the polymer to the saturation, which can slow down the impregnation process.
Figure 7: Mass fraction of ketoprofen ($\omega_{\text{keto}}$) impregnated in polymer filled 625 microcontainers at different temperatures and times: (a) at 100 bar in saturation conditions (b) at 200 bar with a fixed amount of drug dissolved in scCO$_2$(N=3).

Figure 8: Mass of ketoprofen impregnated at 200 bar in 625 microcontainers at different temperatures and times (N=3) as function of the CO$_2$ density.

Table 2: Drug loading of impregnated microcontainers. The drug weight refers to total loaded mass on individual chips (625 microcontainers) and is normalized with respect to the polymer mass in the microwells (N = 3).

<table>
<thead>
<tr>
<th>T(°C)</th>
<th>P(bar)</th>
<th>Time (h)</th>
<th>Loaded drug per chip (mg)</th>
<th>Drug weight fraction (keto/(PVP+keto))</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>100</td>
<td>1</td>
<td>0.33 ± 0.09</td>
<td>0.15 ±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0.58 ± 0.02</td>
<td>0.26 ±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0.53 ± 0.07</td>
<td>0.23 ±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0.58 ± 0.01</td>
<td>0.27 ±0.03</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>1</td>
<td>0.19 ± 0.02</td>
<td>0.10 ±0.01</td>
</tr>
<tr>
<td></td>
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<td>4</td>
<td>0.40 ± 0.04</td>
<td>0.18 ±0.02</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>0.55 ± 0.03</td>
<td>0.26 ±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0.71 ± 0.04</td>
<td>0.27 ±0.03</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
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<td>0.08 ± 0.03</td>
<td>0.04 ±0.01</td>
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<tr>
<td></td>
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<td>0.16 ± 0.04</td>
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<td>4</td>
<td>1.05 ± 0.03</td>
<td>0.33 ±0.01</td>
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</table>
3.4 Raman spectroscopy

Another important aspect for a drug delivery device is the API solid state properties and the stabilization of the amorphous phase in a polymer matrix. For this purpose, Raman scattering spectroscopy was utilized to detect the presence of amorphous ketoprofen and the molecular interactions with the polymer. In order to elucidate the presence of drug-polymer interactions, additional Raman spectra were collected from microcontainers filled with physical mixtures (PMs) of the polymer and drug. Raman scattering spectra of both fresh ketoprofen:PVP PMs and mixtures prepared by supercritical CO$_2$ are shown in Figure 9. In the ketoprofen:PVP PM case, the Raman scattering spectra from both compounds are added together and retain all characteristics of isolated ketoprofen and PVP, see Figure 9(a). To illustrate this, the inter-ring ketoprofen carbonyl [C=O] stretching mode [22] found at 1657 cm$^{-1}$ is examined for different drug/PVP ratios. In all cases the Raman intensity of the ketoprofen C=O stretching mode compared to the 1602 cm$^{-1}$ C-C stretching mode is $I_{1657}/I_{1602} > 1$. The Raman vibration can be fitted to two Lorentzian functions outlining modes that belong to both ketoprofen and PVP. The FWHM of the C=O stretching mode is similar to the one recorded for pure ketoprofen shown in the bottom part of Figure 9(a). The PM result indicates no observable intermolecular interaction between the two compounds [13]. The 1657 cm$^{-1}$ Raman mode for supercritical CO$_2$ impregnated ketoprofen:PVP mixtures behaves differently, i.e. the vibrational mode intensity is reduced and the FWHM is significantly increased, see Figure 9(b). In this case, the two Lorentzian fits yield essentially a single Raman peak without a clear contribution from PVP and $I_{1657}/I_{1602} < 1$ almost for all drug/PVP ratios. Since there is no observable frequency shift, the result suggests that this carbonyl group is not involved in the formation of hydrogen bonds with PVP molecules. The recorded change rather indicates rearrangement of ketoprofen molecules into a less organized solid state, and a similar effect was previously observed for aged ketoprofen:PVP PM mixtures [13]. The presence of amorphous ketoprofen was previously confirmed by X-ray diffraction [13]. It is also expected that the CO$_2$ assisted impregnation of PVP with ketoprofen breaks or reduces the interaction between adjacent ketoprofen molecules and induces the formation of the hydrogen bonds between oxydryl groups of ketoprofen molecules and carbonyl groups of PVP [23].
Figure 9: Raman spectra of (a) physical mixtures of PVP and crystalline ketoprofen in different representative weight percentages and (b) spectra of PVP impregnated with the same ketoprofen weight percentages at different experimental conditions (see table 2).

4. Conclusions

The present study investigates the use of CO$_2$-aided impregnation to load ketoprofen into microcontainers filled with PVP by tuning temperature, pressure, time, and drug concentration in the fluid phase. The characterization study shows that drug loading can be controlled with high accuracy and reproducibility and the impregnated drug is in the amorphous state. At any tested pressure there is an increase of drug loading over time for any tested temperature meaning that after 1 hour the equilibrium between the polymer and the CO$_2$ phases is not yet attained. From the tests at 100 bar it was observed that the loading is strongly dependent on the drug concentration in the impregnating medium. Unexpectedly, in the tests at 200 bar the loading increased with the temperature despite the increasing drug solubility in the scCO$_2$. This was attributed to the predominance of drug diffusivity in the polymer matrix. In contrast, at 200 bar more limited loading gains for increasing impregnation time were obtained probably due to the proximity of drug saturation in the polymer. The Raman scattering spectra of the impregnated matrices indicate a rearrangement of ketoprofen molecules into a less organized state. However, no observable frequency shift in was noticed for the spectral modes typically ascribed to drug-polymer interactions, which suggests that the drug molecule is unlikely to be involved in the formation of hydrogen bonds with PVP molecules.

Our results demonstrate that the combination of powder loading and SCI can be used as a high throughput loading technique for microfabricated devices for oral drug delivery.
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5. Bibliography


